The therapeutic potential of small-molecule modulators of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel

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1.1 INTRODUCTION

The cystic fibrosis transmembrane conductance regulator (CFTR)[1] is an ATP-binding cassette (ABC) transporter.[2] ABC transporters are found in all organisms, where they perform diverse physiological roles. The vast majority of ABC transporters pump assorted substrates across cell membranes either into (importers) or out of (exporters) cells.[2] Some ABC transporters (e.g. the sulphonylurea receptor; SUR1 (ABCC8; pancreas) and SUR2 (ABCC9; heart and muscle)) regulate associated ion channels.[3,4] But, only CFTR (ABCC7) forms an ion channel.[5,6]

CFTR is principally expressed in the apical membrane of epithelia throughout the body lining ducts and tubes.[7] It controls the quantity and composition of epithelial secretions by (i) forming a small-conductance (6-10 pS) anion-selective channel with complex regulation[8,9] and (ii) regulating the function of other ion channels and transporters.[10,11] Thus, CFTR plays a pivotal role in epithelial physiology that influences development, growth, innate immunity and fecundity. The importance of CFTR is dramatically highlighted by three common diseases: cystic fibrosis (CF), secretory diarrhoea and autosomal dominant polycystic kidney disease (ADPKD). CF is caused by loss-of-function mutations in CFTR,[7] whereas secretory diarrhoea and ADPKD are associated with unphysiological CFTR activity.[12,13] However, these are not the only diseases linked to CFTR. Some forms of male infertility, pancreatitis and bronchiectasis are termed CFTR-related disorders because they are caused by mutations that preserve partial CFTR function,[14] while some reproductive disorders involve unphysiological CFTR activity.[15]

Here, we review selectively the therapeutic potential of small-molecule CFTR modulators in the treatment of CF, secretory diarrhoea and ADPKD. We discuss how CFTR's

physiological role and malfunction in disease have informed drug development. We explore the structure and function of CFTR to understand the mechanism of action of small-molecule CFTR modulators. Finally, we consider some of the challenges to be overcome before small-molecule CFTR modulators realise their promise as therapeutics.

1.2 THE PATHOPHYSIOLOGY OF CFTR

1.2.1 The physiology of CFTR

CFTR plays a crucial role in regulating the quantity and composition of epithelial secretions throughout the body. However, examination of the physiology of individual epithelia reveals tissue-specific differences in CFTR function. A variety of factors contribute to this diversity including tissue architecture, the distribution of proteins that interact with CFTR to modulate its expression and function and the role of CFTR as both an anion channel and transport regulator.

Ducts and tubes in the pancreas, intestine, hepatobiliary system and reproductive tissues transport a variety of protein-rich cargoes. For this purpose, these ducts and tubes are lined by epithelia, which secrete fluid and electrolytes to lubricate their surfaces (Fig. 1).[7,16] The process involves the active transcellular movement of Cl^- and the passive paracellular movement of Na^+ and water. A series of ion channels and transporters in the basolateral membrane accumulate actively Cl^- inside epithelial cells. However, CFTR plays the pivotal role by providing an apical membrane pathway for Cl^- to exit passively from cells down a favourable electrochemical gradient and a key point at which to regulate transepithelial fluid and electrolyte movements (Fig. 1). By contrast, in sweat duct epithelia, the coordinated activity of CFTR and the epithelial

Na^+ channel (ENaC) reabsorb salt across a water-impermeable epithelium to generate a hypotonic fluid in the duct lumen that emerges onto skin as sweat (Fig. 1). Interestingly, CFTR is located in both the apical and basolateral membranes of sweat duct epithelia where it provides pathways for passive Cl^- transport across the epithelium following active Na^+ transport.[7,17]

Like sweat duct epithelia, airway epithelia possess both CFTR and ENaC channels in their apical membrane (Fig. 1). They also possess a similar repertoire of basolateral membrane ion channels and transporters to those found in secretory epithelia. Thus, airway epithelia either absorb or secrete fluid and electrolytes depending on the prevailing electrochemical gradient and neurohumoral signals.[7,16,18] The coordinated activity of CFTR and ENaC in airway epithelia generates the thin layer of airway surface liquid (ASL) above which lies a mucus gel that traps debris in inhaled air (Fig. 1). Beating cilia protruding from the apical membrane of airway epithelial cells propel mucus up the airways to remove debris from the lungs (termed mucociliary clearance). CFTR is also expressed in serous cells of submucosal glands in the proximal airways. CFTR-driven fluid and electrolyte secretion by these cells flushes mucins and antimicrobial factors from submucosal glands onto the surface epithelium where they play important roles in mucociliary clearance and host defence.[19]

In the kidney, CFTR is differentially expressed along the length of the nephron,[20] where it serves several functions. First, CFTR acts as a regulated anion channel to transport Cl^- across the apical membrane of epithelial cells in different nephron segments.[21] Second, some data suggest that CFTR co-assembles with the renal outer medullar potassium (ROMK) channel (Kir1.1) to form the ATP-sensitive K^+ channel that recycles K^+ across the apical membrane of

epithelial cells in the thick ascending limb of the loop of Henle during salt reabsorption.[22] Third, CFTR stabilises cubulin expression during receptor-mediated endocytosis in the proximal tubule.[23] Thus, CFTR plays wide-ranging roles in epithelial physiology. Loss of CFTR function or unphysiological CFTR activity has profound consequences for epithelial ion transport and human health. Below, we consider CF, secretory diarrhoea and ADPKD.

1.2.2 Cystic fibrosis

The genetic disease CF is an important medical problem, accounting for most cases of severe chronic obstructive lung disease and exocrine pancreatic dysfunction in Caucasian children and young adults.[7,24] Current therapies for CF are directed against disease symptoms, not its root cause CFTR malfunction. They include physiotherapy, mucolytic drugs and antibiotics to treat lung disease, and pancreatic enzyme replacement therapy and supplemental nutrition to overcome gastrointestinal dysfunction.[7,24] Together with specialist patient care in CF centres, improvements in symptomatic therapy have increased average life expectancy to around 40 years in North America and some European countries.[24]

The wide-ranging manifestations of CF, including chronic lung disease, exocrine pancreatic insufficiency, meconium ileus, male infertility and salty sweat are caused by defective CFTR-mediated fluid and electrolyte movements across epithelia.[7] CFTR malfunction in secretory epithelia prevents their lubrication leading to the stasis of protein-rich cargoes, the obstruction and ultimately destruction of ducts and tubes in organs such as the pancreas, intestine, hepatobiliary system and reproductive tissues. By contrast, loss of CFTR function in CF sweat ducts leads to salty sweat, a hallmark used clinically to diagnose the disease.[17]

The major cause of morbidity and mortality in CF is progressive chronic lung disease. CF lung disease is caused by defective epithelial ion transport, which impairs mucociliary clearance leading to mucus accumulation, airway obstruction and persistent bacterial infections.[7,18,25] In general, there are two mechanisms by which CF mutations cause harm. First, the mutant protein is not delivered to the apical membrane of epithelial cells. Second, the mutant protein is present at the apical membrane, but its function is altered. Both mechanisms are exemplified by the most common CF mutation, F508del, the deletion of a phenylalanine residue at position 508 of the CFTR protein sequence, which is located in the first nucleotide-binding domain (NBD1). About 70% of CF patients carry two copies of the F508del mutation and 90% at least one. The prevalence of the F508del mutation argues strongly that it is the primary target for therapy development.

The molecular basis for the F508del defect is protein misfolding, which causes retention of the mutant protein in the endoplasmic reticulum and its subsequent degradation by the proteasome.[26-28] The vast majority of F508del-CFTR therefore fails to reach its correct cellular location. Worse, the little that reaches the apical membrane is unstable, residing there for < 4 h compared with > 24 h for wild-type CFTR.[29] The F508del-CFTR mutation also disrupts severely CFTR channel gating (Fig. 2).[30] For wild-type CFTR, channel openings occur frequently and are separated by short periods of channel closure (Fig. 2). By contrast, F508del-CFTR channel openings occur infrequently and are separated by prolonged periods of channel closure (Fig. 2).

In airway epithelia, loss of CFTR-driven transepithelial fluid and electrolyte transport has three main consequences. First, defective HCO_3^- secretion prevents secreted mucins expanding to form mucus-gel networks; instead they remain aggregated and difficult to transport.[31] Second, CFTR malfunction in submucosal glands disrupts the secretion of fluid, mucins and anti-microbial factors onto the surface epithelium.[19] Third, CFTR malfunction in the surface epithelium depletes ASL.[18] This latter defect highlights the dual roles of CFTR as a Cl^- channel and transport regulator. Loss of CFTR Cl^- channel function prevents fluid and electrolyte secretion onto the surface epithelium, whereas dysregulation of ENaC by CFTR leads to excessive absorption of Na^+ and fluid, dehydrating ASL.[18] Loss of ASL impairs mucociliary clearance because cilia are unable to beat effectively on dehydrated airway surfaces. Mucus accumulation causes airway obstruction. Moreover, defective mucus transport results in persistent infection of CF airways by microbial pathogens (e.g. *Pseudomonas aeruginosa*).[7] Bacterial infections trigger inflammatory responses in CF airways that are excessive in magnitude and protracted in duration. Together, infection and inflammation play a central role in the destruction of lung tissue in CF, leading to bronchiectasis, emphysema and end-stage lung disease.

1.2.3 Secretory diarrhoea

The commonest cause of secretory diarrhoea is infection of the small intestine by enterotoxin-producing bacteria most notably *Vibrio cholerae* and *Escherichia coli*.[12] These bacteria do not damage the intestinal epithelium. Instead, they cause disease by modifying profoundly transepithelial ion transport in the small intestine. For example, it is not unknown for cholera patients to expel more stools than their own body weight.[32] Contamination of water

supplies with enterotoxin-producing bacteria is a major global health problem, causing the death of several million lives annually, mostly young children in underdeveloped countries.[33] However, these organisms, especially *Escherichia coli*, also cause disease in developed countries, particularly among vulnerable young and elderly populations.

Cholera toxin, the major toxin secreted by *Vibrio cholerae* and *Escherichia coli* heat labile toxin have identical cellular mechanisms of action, targeting adenylyl cyclase in intestinal epithelial cells to activate the cAMP signaling cascade leading to the phosphorylation of target proteins by protein kinase A (PKA).[12] By contrast, *Escherichia coli* heat stable toxin activates guanylate cyclase and hence, cGMP-dependent protein kinase (PKG) in intestinal epithelial cells.[34] Activation of the cAMP signaling cascade in the small intestine stimulates CFTR-mediated transepithelial Cl^- secretion by crypt epithelial cells.[12] It also inhibits electroneutral salt reabsorption by villus epithelial cells in the small intestine and stimulates K^+ secretion by crypt epithelial cells in the colon.[12] Like PKA, PKG phosphorylates CFTR leading to the activation of CFTR-mediated transepithelial Cl^- secretion by crypt epithelial cells in the small intestine.[35] Because Na^+-coupled glucose absorption by villus epithelial cells is unaffected by PKA and PKG, oral rehydration therapy (ORT) with isosmolar sodium glucose solutions is the treatment of choice for secretory diarrhoea in all countries.[36] Although ORT effectively rehydrates afflicted patients, it does not lessen the diarrhoea.[12] For this reason, pharmacological interventions to prevent diarrhoea (e.g. small-molecule CFTR inhibitors) would be a welcome adjuvant to ORT.

1.2.4 Autosomal dominant polycystic kidney disease

ADPKD is the most common single gene disorder to affect kidney function, with an incidence of approximately 1 in 1000 live births in all ethnic groups.[37] The disease is caused by mutations in the polycystin proteins (polycystin-1 and polycystin-2) that initiate a cascade of events, which result in the formation of multiple epithelial cysts containing a fluid-filled cavity surrounded by a single layer of immature renal epithelial cells.[37-39] The development and growth of cysts in ADPKD is accelerated by activation of the cAMP signal transduction cascade, which stimulates the proliferation of ADPKD epithelial cells and fluid accumulation within the cyst lumen powered by CFTR-mediated transepithelial Cl^- secretion.[13,40] The insidious formation and growth of multiple ADPKD cysts progressively destroys kidney function leading to severe renal failure.[37,38] At present, there are no cures for ADPKD, which accounts for approximately 7-10% of patients requiring kidney transplantation and dialysis.[38] Among the many approaches now under consideration to develop rational new therapies for ADPKD,[37] one strategy is to prevent fluid accumulation within ADPKD cysts using small-molecule CFTR inhibitors.

1.3 CFTR STRUCTURE AND FUNCTION

CFTR has a modular design composed of two membrane-spanning domains (MSDs), two nucleotide-binding domains (NBDs) and a unique regulatory domain (RD).[8,9] The MSDs are composed of six transmembrane segments linked by short extracellular, but long intracellular loops. The NBDs possess conserved ATP-binding motifs (ABC signature (LSGGQ) sequence and Walker motifs). The RD is distinguished by multiple consensus phosphorylation sites and many charged residues.

Initial efforts to investigate CFTR structure and function utilised functional studies of CFTR constructs bearing site-directed mutations. This work demonstrated that CFTR is a small conductance anion-selective channel with complex regulation. The MSDs form an anion-selective pore, but instead of multiple transmembrane segments governing conduction and permeation, the sixth transmembrane segment (M6) plays a dominant role.[8,41] Phosphorylation of the RD by PKA at multiple consensus sites is a prerequisite for CFTR activity.[8,42] ATP binding and hydrolysis at the NBDs control the opening and closing of the CFTR pore.[8,42] But, detailed mechanistic insight has for a long time been lacking.

The elucidation of crystal structures of isolated NBDs (e.g. CFTR NBD1)[43] and intact ABC transporters, especially Sav1866, the multidrug transporter of *Staphylococcus aureus*,[44] revolutionised understanding of CFTR structure and function. They revealed that the NBDs function as a head-to-tail dimer with two ATP-binding sites located at the dimer interface (Fig. 3). One ATP-binding site is formed by the Walker A and B motifs of NBD1 and the LSGGQ motif of NBD2 (termed site 1), while the other is formed by the Walker A and B motifs of NBD2 and the LSGGQ motif of NBD1 (termed site 2) (Fig. 3).[43] Structural studies also illuminated the impact of CF mutations on CFTR function. For example, F508, the site of the commonest CF mutation, is located remote from the NBD dimer interface on the surface of NBD1 in a prime position to interact with the MSDs (Fig. 3).[43] By contrast, other CF mutations (e.g. G551D and G1349D) affect critical residues in the two ATP-binding sites (Fig. 3), providing explanations for the deleterious effects of these mutations on CFTR channel gating.[45,46]

Vergani et al.[47] integrated biochemical (e.g. Aleksandrov et al.[48]) and functional (e.g. Zeltwanger et al.[49]) data with the results of structural studies of ABC transporters to propose the ATP-driven NBD dimerisation model of CFTR channel gating. In this model, CFTR channel gating is controlled by ATP binding and hydrolysis at site 2 driving cycles of NBD dimer assembly and disassembly. In support of this model, R555 (NBD1) and T1246 (NBD2), two residues on either side of ATP-binding site 2 are energetically coupled in open, but not closed channels, arguing that the NBDs change their conformation during channel gating.[50] However, subsequent studies revealed that ATP hydrolysis at ATP-binding site 2 leads to partial, not full separation of the NBD dimer.[51,52] Thus, during the gating cycle, ATP-binding site 1 remains tightly closed around a bound ATP molecule, whereas ATP-binding site 2 undergoes conformational changes, closing following binding of an ATP molecule to form the NBD dimer, but opening again after ATP cleavage to release the hydrolytic products.

Dynamic reorganization of the NBDs in turn power conformational changes of the MSDs that gate the CFTR pore to control anion flow. Studies of CF mutants (e.g. Cotton et al.[53]) had suggested that the intracellular loops (ICLs) which connect transmembrane segments within the MSDs play a crucial role in coupling the NBDs to the CFTR pore. However, confirmation required the development of structural models of CFTR by Serohijos et al.[54] and Mornon et al..[55] In these structural models, each ICL consisted of two long α-helical extensions of transmembrane segments with an intervening short α-helix at its most cytoplasmic location orientated parallel to the plane of the membrane. Because this short α-helix interacted with the NBDs, it was termed the coupling helix.[54,55] Several important conclusions about NBD-MSD communication are suggested by the structural models of Serohijos et al.[54] and Mornon et al..[55]

First, the ICLs communicate with both the same and the opposite NBD (e.g. ICL1 (MSD1) with NBD1 and ICL2 (MSD1) with NBD2). Second, the coupling helices of ICL1 and ICL3 interact directly with ATP-binding sites 1 and 2, respectively. Third, the coupling helix of ICL4 interacts with F508. Thus, the F508del mutation affects a critical interface in the CFTR Cl^- channel.

Electron crystallography of two-dimensional CFTR crystals suggested that the CFTR pore resembles an asymmetric hour-glass, with a deep wide intracellular vestibule and a shallow extracellular vestibule separated by a constriction.[56] This image of the CFTR pore demonstrates striking agreement with predictions from functional studies.[8,41] Building on these studies, Norimatsu et al.[57] used cysteine scanning mutagenesis to test the predicted orientation of amino acid side-chains in M3, M6, M9 and M12 from a Sav1866-based molecular model of the CFTR pore. The authors' data suggest that the constriction in the CFTR pore is located towards the extracellular end of the channel with the boundary between the outer vestibule and the constriction located near T338 (M6) and I1131 (M12) and that between the constriction and the inner vestibule located near S341 (M6) and T1134 (M12). Functional evidence argue that the pore constriction determines the anion selectivity of the CFTR,[58] exhibiting characteristics of a lyotropic series (i.e. anions with the lowest free energy of hydration enter first and bind strongest).[59] In support of the degraded ABC transporter model of CFTR function,[60] the constriction is also a plausible location of the gate, which controls anion flow through the CFTR pore.[61] However, other studies argue that the gate might occupy a more cytoplasmic location within the CFTR pore.[62] Identifying the location of this gate is crucial to understand the CFTR gating pathway, the sequence of conformation changes initiated by ATP binding to the NBDs that lead to Cl^- flow through the CFTR pore. In turn, this knowledge as well as information

about the architecture of the CFTR pore is vital to understand the mechanism of action of small-molecule CFTR modulators.

1.4 RESTORATION OF CFTR FUNCTION

To target the root cause of CF, future therapies should (i) overcome the F508del-CFTR processing defect and traffic the mutant protein to the apical membrane;[26] (ii) extend the residence time of F508del-CFTR at the apical membrane[29] and attenuate channel "rundown" (e.g. Schultz et al.[63]) and (iii) rescue the defective channel gating of F508del-CFTR.[30] Thus, small-molecules with two, possibly three types of activity are required to suppress the deficits in F508del-CFTR expression and function.

Some CFTR modulators (e.g. genistein)[64] were identified using conventional assays of CFTR function. However, by far the most successful strategy is high-throughput screening (HTS).[65] HTS exploits a reliable, sensitive, cost-effective assay to screen libraries of chemically diverse small-molecules to identify lead compounds for medicinal chemistry optimisation. For further information about the use of HTS to discover small-molecule CFTR modulators, see Verkman & Galietta.[65] Below, we discuss selectively, small-molecules that rescue the expression and function of F508del-CFTR.

1.4.1 CFTR correctors

Small-molecules that overcome the processing defect of F508del-CFTR to rescue its cell surface expression are termed CFTR correctors.[65,66] CFTR correctors might interact directly with CFTR, by acting as substrate mimics or active site inhibitors. Alternatively, they might

target one or more of the many CFTR interacting proteins that orchestrate and control CFTR processing, its delivery to, and expression at the apical membrane. This latter group of CFTR correctors is termed proteostasis regulators because they aim to treat CF by manipulating the concentration, conformation, quaternary structure and/or location of CFTR.[67]

The Cystic Fibrosis Foundation (Bethesda, USA), a nonprofit, donor-supported organization, developed a new model for supporting drug development (termed Venture Philanthropy) when it funded Aurora Biosciences then Vertex Pharmaceuticals to develop small-molecule therapies that target directly CF mutants. The first CFTR corrector identified by Vertex Pharmaceuticals was the quinazoline VRT-325, which restored CFTR function to CF bronchial epithelia expressing F508del-CFTR (F508del-CFBE) to ~10% that of human bronchial epithelia expressing wild-type CFTR (HBE),[68] an amount similar to that achieved by the bisaminomethylbithiazole CFTR corrector corr-4a.[69] Subsequently, Vertex Pharmaceuticals developed VX-809 (Fig. 4), a potent, selective and orally bioavailable CFTR corrector that partially restores CFTR expression and function to F508del-CFBE.[70] Treatment of cells expressing recombinant F508del-CFTR with VX-809 (3 μM) for 24 h abrogated the protease-sensitivity of mutant protein and accelerated its conversion from the immature, core-glycosylated form (band B) to the mature, fully-glycosylated form (band C).[70] These data suggest that VX-809 promotes the correct folding and intracellular transport of a fraction of F508del-CFTR protein. Strikingly, following treatment with VX-809 (3 μM for 24 - 48 h), F508del-CFTR Cl^- channels exhibited the same open probability (P_o) as wild-type CFTR, arguing that VX-809 normalises the channel function of F508del-CFTR.[70] Finally, when compared with other CFTR

correctors VX-809 demonstrated improved selectivity and greater efficacy, achieving 14% HBE function restored to F508del-CFBE.[70]

Based on the success of preclinical studies, VX-809 was tested in 89 adult CF patients homozygous for F508del-CFTR in a 28-day randomised, double-blind placebo-controlled (phase IIa) clinical trial.[71] Although VX-809 was well tolerated by CF patients and caused a modest reduction in sweat Cl^- concentration , it was without effect on patient-reported outcomes, lung function (measured by forced expiratory volume in one second, FEV_1) and nasal potential difference (NPD; a measure of CFTR function in the nasal epithelium).[71]

A potential explanation for the lack of clinical efficacy of VX-809 is provided by two recent studies.[72,73] Mendoza et al.[72] identified an apparent efficacy ceiling among CFTR correctors that likely interact directly with F508del-CFTR: none had potency < 1 μM nor efficacy > 15% wild-type CFTR function. To elucidate the mechanistic basis of this efficacy ceiling, the authors identified amino acid positions statistically-coupled to position 508 in ABC transporter sequences and evaluated the impact of mutations at these positions on NBD1 folding and CFTR protein maturation.[72] Consistent with previous results (e.g. Serohijos et al.[54] and Thibodeau et al.[74]), F508del altered both NBD1 folding and the interaction of NBD1 with ICL4. Mutations that corrected either defect alone restored partially F508del-CFTR expression and function. Only combinations of mutations that rescued NBD1 folding and stabilised the interaction of NBD1 with ICL4 restored F508del-CFTR expression and function to wild-type levels.[72] Identical conclusions were obtained by Rabeh et al.[73] investigating the thermodynamics of NBD1 folding and the stability of the NBD1:ICL4 domain interface. Taken together, the

results of these studies provide a framework to identify efficacious CFTR correctors that restore wild-type expression and function to F508del-CFTR.

1.4.2 CFTR potentiators

Small-molecules that repair the F508del-CFTR gating defect are termed CFTR potentiators (not openers or activators because they do not open silent CFTR Cl^- channels).[65] They interact directly with CFTR to enhance channel gating following PKA-dependent phosphorylation. Some CFTR potentiators increase the frequency of channel opening (e.g. dichlorofluorescein) (Z Cai and DN Sheppard, unpublished observation), other CFTR potentiators prolong the duration of channel openings (e.g. phloxine B)[75] and yet others increase both the frequency and duration of channel openings (e.g. UC_{CF}-853)[76] (Y Wang, Z Cai and DN Sheppard, unpublished observation) (Fig. 5).

The CFTR potentiator that has received greatest attention is the isoflavone genistein.[64,77] In the presence of a maximally effective concentration of the cAMP agonist forskolin (10 μM), genistein (50 μM) robustly enhanced F508del-CFTR activity following its delivery to the cell surface by low-temperature incubation (e.g. Hwang et al.[78]). Genistein augments strongly F508del-CFTR channel activity by accelerating the rate and prolonging the duration of channel openings.[77] Using the ATP-driven NBD dimerisation model of CFTR channel gating,[47,50] Ai et al.[79] speculated that genistein might bind at the NBD dimer interface and promote channel opening by lowering the free energy of the transition state. The authors also suggested that genistein might slow the rate of channel closure by stabilising the NBD dimer in its ATP bound configuration.[79] Consistent with these ideas, Moran et al.[80] used virtual ligand docking with a

molecular model of the NBD dimer to suggest that genistein binds at the dimer interface (Fig. 6). The genistein-binding site is distinct from the two ATP-binding sites of CFTR.[81,82] It is composed of sequences from both NBD1 (Walker A, Walker B and LSGGQ) and NBD2 (LSGGQ), with those from NBD1 forming a cavity in which genistein docks.[80] Because of its efficacy *in vitro*, Illek et al.[83] tested genistein on CF patients bearing the G551D mutation, a relatively common CFTR mutation that is without effect on protein processing, but severely disrupts channel gating.[46] Of note, application of genistein (50 μM) to the nasal epithelium of CF patients with the G551D mutation restored 15% of wild-type CFTR function (measured by NPD).[83] However, genistein's lack of potency and selectivity precludes its application in the clinic.

In 2012, ivacaftor (Kalydeco™; VX-770) (Fig. 4), a potent, selective and orally bioavailable CFTR potentiator developed by Vertex Pharmaceuticals, became the first small-molecule targeting CFTR to be approved for patient use (www.cff.org/aboutCFFoundation/NewsEvents/1-31-FDA-Approves-Kalydeco.cfm). It was developed from a chemical scaffold identified as a CFTR potentiator in the second round of HTS conducted by Vertex Pharmaceuticals to identify small-molecule CFTR modulators.[68,84] By increasing the frequency and duration of channel openings, ivacaftor (1 μM) restored F508del-CFTR channel activity to wild-type levels (measured by P_o). Treatment of F508del-CFBE with ivacaftor (10 μM) increased CFTR-mediated transepithelial ion transport (measured by short-circuit current (I_{sc})) to about half those of HBE.[84] Consistent with these data, ivacaftor (10 μM) increased ASL volume and ciliary beat frequency of F508del-CFBE to about half those of

HBE.[84] These data suggest that ivacaftor restores partially CFTR function and rescues transepithelial ion transport by F508del-CFBE.

In its initial (phase II) clinical trial, ivacaftor was tested in 39 adult CF patients carrying the G551D-CFTR mutation.[85] The patients took ivacaftor orally in 14- or 28-day blinded, randomised, placebo-controlled trials. Ivacaftor was well tolerated by CF patients.[85] More importantly, however, in CF patients receiving ivacaftor (150 mg twice daily) sweat Cl^- concentration decreased by ~40 mmol/l to approach the normal range (< 60 mmol/l).[85] At this dose, lung function (measured by FEV_1) increased by 9%.[85] A similar magnitude increase in lung function (measured by FEV_1) was observed in the phase III clinical trial of ivacaftor, which involved 161 adult CF patients with the G551D-CFTR mutation.[86] For three reasons, the results of this clinical trial were highly significant for CF patient care. First, lung function increased rapidly within 2 weeks of commencing therapy and was sustained through the 48 weeks of the trial.[86] Second, in comparison with placebo-treated patients, ivacaftor-treated patients were 55% less likely to experience a pulmonary exacerbation over the 48 week period, suggesting that ivacaftor improved lung defence against bacterial infections.[86] Third, ivacaftor-treated patients gained significant weight (~2.7 kg) over the trial period, suggesting that ivacaftor improves intestinal function resulting in better absorption of nutrients.[86] When ivacaftor was tested in CF children aged 6 – 11 years with at least one copy of the G551D mutation, very similar results were obtained. Lung function improved, weight was gained and sweat Cl^- concentration fell.[87] Based on the results of these trials, the FDA and EMEA approved ivacaftor for use in CF patients aged 6 years and older with one copy of the G551D mutation (www.cff.org/aboutCFFoundation/NewsEvents/1-31-FDA-Approves-Kalydeco.cfm).

Finally, recent work suggests that ivacaftor might potentiate CFTR channel gating by a mechanism distinct from that of CFTR potentiators, which enhance ATP-dependent channel gating.[79] Using purified, reconstituted recombinant CFTR protein, Eckford et al.[88] demonstrated that ivacaftor opens CFTR channels following PKA-dependent phosphorylation, in the absence of ATP. These and other data,[88] suggest that ivacaftor enhances ATP-independent CFTR channel gating,[89] such as that exhibited by the G551D-CFTR mutant.[46] They further suggest that CFTR channel gating can be potentiated by multiple mechanisms. However, the observation that ivacaftor potentiates multiple CF mutants,[90] including some that do not disrupt CFTR channel gating,[91] argues that it and related small-molecules will have widespread utility in the future treatment of CF patients.

1.4.3 CFTR corrector-potentiators

Because F508del-CFTR disrupts both the expression and function of CFTR,[26,30] the ideal small-molecule therapy for CF patients bearing this mutation would combine the properties of CFTR correctors with those of CFTR potentiators. Consistent with this idea, Van Goor et al.[68] demonstrated that the effects of the CFTR potentiator VRT-532 on F508del-CFBE were synergistic with those of the CFTR corrector VRT-325. Together VRT-325 and VRT-532 generated levels of CFTR-mediated transepithelial ion transport in F508del-CFBE > 20% those in HBE.[68] Similar results were observed using the CFTR corrector VX-809 and the CFTR potentiator ivacaftor.[70] Moreover, in clinical trials both small-molecules together restored significant levels of lung function to some CF patients homozygous for F508del-CFTR (http://investors.vrtx.com/releasedetail.cfm?ReleaseID=677520), whereas by themselves neither

VX-809 nor ivacaftor improved the lung function of these CF patients.[71,92] These data argue that dual-acting small-molecules, possessing corrector and potentiator activity (so called CFTR corrector-potentiators) have significant therapeutic potential. But, could such molecules be discovered or designed?

The first two CFTR corrector-potentiators identified were discovered serendipitously. First, Wang et al.[93] demonstrated that the CFTR potentiator VRT-532, by itself, rescues the misprocessing of F508del-CFTR. Building on these data, Wellhauser et al.[94] showed that VRT-532 reduces the ATPase activity of F508del-CFTR and abrogates its protease susceptibility. The authors' data suggest that VRT-532 binds directly to F508del-CFTR to induce a change in conformation that improves its stability.[94] They also suggest that VRT-532 enhances channel gating by slowing ATP hydrolysis at ATP-binding site 2.[94] Second, Pedemonte et al.[95] discovered that the aminoarylthiazole corr-2b, one of the first CFTR correctors identified by HTS,[69] exhibits dual activity as both a CFTR corrector and a CFTR potentiator. When compared with small-molecules that act as CFTR correctors alone, (e.g. the bisaminomethylbithiazole corr-4a), corr-2b generated double the amount of forskolin-stimulated CFTR Cl^- current (I_{FSK}) relative to the total CFTR Cl^- current measured in the presence of forskolin and the CFTR potentiator genistein (I_{TOT}) (corr-4a: I_{FSK}/I_{TOT} ~40%; corr-2b: I_{FSK}/I_{TOT} ~80%).[95] Interestingly, aminoarylthiazoles are atypical CFTR potentiators; they do not act acutely, but instead require protein synthesis to exert their effects.[95]

In search of dual-acting small-molecules to rescue F508del-CFTR, Verkman and colleagues screened ~110,000 drug-like chemicals with their HTS assay.[96] From this screen,

Phuan et al.[96] identified cyanoquinolines, a chemical scaffold with independent CFTR corrector and potentiator activity. One cyanoquinoline, CoPo-22 trafficked F508del-CFTR to the cell surface with comparable potency to corr-4a and potentiated F508del-CFTR channel activity with potency similar to genistein.[96] Structure-activity relationship studies revealed that the corrector and potentiator activities of cyanoquinolines could be dissociated from each other.[96] Molecular modeling suggested that cyanoquinolines require both a flexible diamine tether and a short bridge between the cyanoquinoline and arylamide moieties for optimal F508del-CFTR corrector and potentiator activities.[97] Phuan et al.[96] speculated that separation of corrector and potentiator activities might be achieved by distinct binding sites on F508del-CFTR and/or CFTR-interacting proteins. By contrast, Knapp et al.[97] posited the imaginative idea of a single binding site that alters its conformation during the processing of F508del-CFTR and its delivery to the cell surface. Finally, Kalid et al.[98] adopted *in silico* structure-based screening to search for CFTR correctors, potentiators and dual-acting small molecules. Based on analyses of homology modeling, three binding sites for small-molecules were identified on F508del-CFTR: (i) the NBD1:NBD2 interface, (ii) the NBD1:ICL4 cavity formed by deletion of F508 and (iii) the interface of NBD1 and NBD2 with ICL1, 2 and 4 (Fig. 6).[98] From 100,000 chemicals screened *in silico* and 496 selected for functional testing, two CFTR corrector-potentiators were identified. EPX-108380 docked at the NBD1:NBD2 interface, while EPX-107979 docked at the NBD1:NBD2:ICL1, 2 and 4 interface.[98] This study demonstrates convincingly the utility of *in silico* structure-based screening to search for CFTR modulators.

1.4.4 Towards the therapeutic application of CFTR correctors and potentiators

The application of HTS to CFTR drug discovery has led to the identification of abundant small-molecules that rescue the expression and function of CF mutants.[65] The pressing challenge now is to transform discoveries in the laboratory into effective therapies for CF patients. The pioneering efforts of Vertex Pharmaceuticals especially the success of ivacaftor demonstrate convincingly the value of targeting directly CFTR with small-molecules. However, they urge further prompt progress to develop CFTR correctors and potentiators for CF patients with the F508del mutation, the largest group of CF patients.

The amount of function that must be restored to individual CF mutants to achieve therapeutic benefit is currently unknown. Some attempts have been made to address this fundamental question by analysing published data on the relationship between the genotype of CF patients, their clinical phenotype and the effects of CF mutants on the CFTR Cl^- channel. For example, Van Goor et al.[68] speculated that restoration of 5 – 30% of wild-type CFTR function to CF patients bearing the F508del mutation would be of therapeutic benefit. Using biochemical and functional data and the relationship $I^{CFTR} = N \times i \times P_o$ (I^{CFTR}, CFTR-mediated apical membrane Cl^- current; N, the number of CFTR Cl^- channels in the apical membrane; i, the amount of current that flows through an open CFTR Cl^- channel and P_o, a measure of its activity), it is possible to predict how much function different therapeutic strategies restore to CF mutants *in vitro*.[99] By contrast, it is not possible to quantify CFTR function *in vivo* using currently available research tools (e.g. NPD), while measures of lung function (e.g. FEV_1) lack sensitivity during the crucial early stages of lung disease. These considerations argue that improvements in disease biomarkers and therapeutic end-points are required to determine how much CFTR function is required for therapeutic benefit.

A further obstacle for CF drug development is the use of animal models to demonstrate therapeutic proof of principle. The failure of mouse models of CF to recapitulate the clinical features of CF[100] limits their utility in preclinical studies of experimental therapeutics. Furthermore, the lack of effects of CFTR potentiators on murine CFTR,[101,102] argues that these agents should not be tested in CF mice. To overcome these difficulties, Vertex Pharmaceuticals employed well-differentiated primary cultures of human airway epithelia grown at an air-liquid interface for therapeutic proof of principle studies.[84] The success of this approach argues that primary cultures of human airway epithelia are the model system of choice for therapeutic proof of principle studies. However, the emergence of animal models that emulate the clinical features of CF (e.g. F508del-CFTR pigs),[103] argues that testing small-molecule therapies in animal models should now be reevaluated.

Finally, the prospects of designing rationally better CFTR correctors and potentiators beckons. The binding sites of some CFTR correctors, potentiators and dual-acting molecules are beginning to be revealed.[98] Precise mapping of these sites will require further understanding of CFTR structure and how it is perturbed by CF mutations. Advances in the structural biology of CFTR (e.g. Rosenberg et al.[104]) and modeling CFTR using the structures of related ABC transporters and knowledge of CFTR function (e.g. Norimatsu et al.[57] and Mornon et al.[105]) argue that rational design might be enhanced. Such therapeutics will likely have improved efficacy and specificity.

1.5 INHIBITION OF CFTR FUNCTION

Secretory diarrhoea, ADPKD and some reproductive disorders are characterised by unphysiological CFTR-mediated transepithelial fluid and electrolyte movements.[12,13,15] The cellular mechanism of CFTR-mediated transepithelial ion transport suggests a number of targets for pharmacological intervention to prevent unphysiological fluid and electrolyte movements. These targets include the basolateral membrane ion channels and transporters that accumulation Cl^- within epithelial cells, signaling molecules that regulate CFTR activity and CFTR, itself. There are two general mechanisms by which small-molecules inhibit directly the CFTR Cl^- channel: allosteric inhibition (e.g. $CFTR_{inh}$-172)[106,107] and open-channel blockade (e.g. glibenclamide).[108] Agents that act as allosteric inhibitors bind to CFTR and interfere with channel gating to hinder Cl^- entry into the channel pore (Fig. 7). By contrast, ligands that act as open-channel blockers bind within the open channel pore to obstruct transmembrane Cl^- flow (Fig. 7).

Using conventional assays of CFTR function, many chemically diverse small-molecule CFTR inhibitor have been identified (for review, see Hwang & Sheppard[77] and Schultz et al.[109]). However, few of these agents are high-affinity CFTR inhibitors and none demonstrate specificity. Allosteric inhibitors interact with other targets within cells at concentrations similar to those that inhibit CFTR, while open-channel blockers of CFTR invariably block other types of Cl^- channels.

1.5.1 CFTR inhibitors and secretory diarrhoea

In search of lead compounds to develop therapeutically-active CFTR inhibitors for secretory diarrhoea, Alan Verkman deployed the HTS assay used with great success to identify

CFTR potentiators.[110] By screening 50,000 compounds, Ma et al.[106] identified the thiazolidinone $CFTR_{inh}$-172 (Fig. 8). $CFTR_{inh}$-172 has several highly desirable properties as a CFTR inhibitor. First, potency: $CFTR_{inh}$-172 reversibly inhibited CFTR Cl^- currents with half-maximal inhibitory potency (K_i) of ~300 nM, an increase in potency of almost 500-fold compared with the widely used CFTR inhibitor glibenclamide.[106] Second, specificity: $CFTR_{inh}$-172 was without effect on several epithelial ion channels and transporters, including Ca^{2+}-activated and volume-regulated Cl^- channels and the ABC transporter P-glycoprotein.[106] Third, efficacy: $CFTR_{inh}$-172 inhibited cholera toxin-induced fluid secretion in the small intestine of mice, highlighting its value as a lead compound for drug discovery.[106] Although $CFTR_{inh}$-172 has the drawbacks of limited water solubility (~20 μM) and reduced potency in intact cells and tissues (K_i ~5 μM),[111] it has become a very widely used research tool. To date, it remains the most selective CFTR inhibitor available.

Early studies to identify the mechanism of action of $CFTR_{inh}$-172 demonstrated that it interfered with channel gating without altering single-channel conductance.[112] These data suggested that $CFTR_{inh}$-172 might act as an allosteric inhibitor of CFTR. However, its mechanism of action is distinct from other allosteric inhibitors (e.g. genistein)[113,114] that interfere with the control of CFTR gating by ATP-driven NBD dimerisation by competing with ATP for binding to ATP-binding site 2. First, Kopeikin et al.[107] demonstrated that CFTR inhibition by $CFTR_{inh}$-172 occurs from either the open or closed state and does not involve disassembly of the NBD dimer, but instead is reminiscent of the inactivation mechanism of voltage-gated cation channels. Second, Taddei et al.[112] found that the CF mutation F508del has increased sensitivity to $CFTR_{inh}$-172 compared to wild-type CFTR, while Caci et al.[115] showed that mutation of R347,

a non-pore lining residue located at the intracellular end of M6, attenuated markedly the inhibitory potency of $CFTR_{inh}$-172. Taken together, these data raise the possibility that $CFTR_{inh}$-172 might interfere with coupling between the NBD dimer and the channel pore.

Following the identification of $CFTR_{inh}$-172, Verkman and colleagues screened 100,000 small-molecules selected for chemical diversity and drug-like properties.[111] Among the four new classes of CFTR inhibitors identified by the authors, the glycine hydrazides attracted special attention because of their unusual mechanism of action. Previous work had demonstrated that open-channel blockers inhibit CFTR by occluding the deep wide intracellular vestibule of the CFTR pore and preventing outwardly directed Cl^- flow through the channel.[77] By contrast, Muanprasat et al.[111] revealed that glycine hydrazides, such as GlyH-101 (Fig. 8), are open-channel blockers that occlude the extracellular vestibule of the CFTR Cl^- channel. When GlyH-101 binds within the extracellular vestibule, Cl^- flow from the extra- to the intracellular side of the membrane is more strongly attenuated than that in the opposite direction. Recently, Norimatsu et al.[116] used induced-fit virtual ligand docking and site-directed mutagenesis to identify the GlyH-101-binding site. The authors' data argue that GlyH-101 occludes the CFTR pore constriction with its hydrophobic naphthalene tail binding in the vicinity of F342 and its negatively charged benzene ring interacting with F337 and T338. These data provide a compelling explanation why GlyH-101 is such an effective open-channel blocker of CFTR.

Like $CFTR_{inh}$-172,[106] GlyH-101 strongly inhibited cholera toxin-induced fluid secretion in the small intestine of mice.[111] However, in marked contrast to $CFTR_{inh}$-172, which must be administered intraperitoneally,[106] GlyH-101 was active when added directly to the lumen of the

small intestine.[111] These data argue that GlyH-101 and related compounds might be developed into a non-absorbable drug therapy for secretory diarrhoea. Towards this goal, Sonawane et al.[117] synthesized a series of malonic acid dihydrazides (MalH) linked to polar moieties and polyethylene glycol (PEG)-coupled butyric acid hydrazides [Gly-$(PEG)_n$] and demonstrated the therapeutic potential of these small-molecules for secretory diarrhoea using a mouse model of cholera toxin-induced fluid secretion. To improve the potency and efficacy of their MalH-based CFTR inhibitors, Verkman and colleagues adopted two approaches. First, chemical conjugation with lectins to form MalH-lectins.[118] Second, formation of divalent, macromolecular MalH conjugates (MalH-PEG-MalH).[119] Of note, the small-molecules developed by Verkman and colleagues[118,119] possess a number of important properties that make them excellent lead compounds for secretory diarrhoea therapy development. These include high water solubility; low toxicity; potent inhibition of CFTR and cholera toxin-induced intestinal fluid secretion; efficacy when delivered directly into the intestine; minimum absorption by intestinal epithelial cells, but stable binding to the glycocalyx covering intestinal epithelial cells to resist drug washout.

The data of Sonawane et al.[119] suggest that non-absorbable divalent polyethylene glycol-malonic acid hydrazides are likely to be especially valuable in the development of rational new therapies for enterotoxin-induced secretory diarrhoea. However, the cost of such therapies might preclude their widespread use in developing countries, where treatment costs should be around US$1 per patient per day.[120] Nevertheless, One World Health (San Francisco, California, USA) in collaboration with BioFocus (Saffron Walden, Essex, UK) have developed glycine hydrazide derivatives where the labile acyl hydrazone is substituted with a stable heterocycle (e.g.

iOWH032; Fig. 8).[120] Based on the success of preclinical studies, clinical trials of iOWH032 began in 2011.[120]

1.5.2 CFTR inhibitors and ADPKD

Hanaoka & Guggino[40] first explored the therapeutic potential of CFTR inhibitors in ADPKD. The authors demonstrated that diphenylamine-2-carboxylate (DPC)[121] and glibenclamide,[108] two non-specific CFTR inhibitors, diminished ADPKD cyst growth *in vitro*, whereas 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid (DIDS), which inhibits other types of epithelial Cl^- channels, but not CFTR, when added to the outside of cells[109] was without effect. Building on these data Li et al.[122] used Madin Darby canine kidney (MDCK) cells as a model system to explore the effects of ion transport inhibitors on renal cyst formation and growth. The data demonstrate that renal cyst growth is retarded by: (i) small-molecules that inhibit directly CFTR by allosteric and open-channel blockade mechanisms; (ii) agents that act indirectly by inhibiting PKA-dependent phosphorylation of CFTR and (iii) chemicals that interfere with the basolateral membrane ion channels and transporters responsible for Cl^- accumulation within epithelial cells.[122] Two important conclusions can be drawn from the data of Li et al..[122] First, the pharmacology of renal cyst growth resembles that of the CFTR Cl^- channel. Second, inhibition of cyst growth by CFTR blockers is correlated with blockade of cAMP-stimulated transepithelial Cl^- secretion, not cell proliferation.

As for secretory diarrhoea, the therapeutic potential of CFTR inhibitors in ADPKD has been strengthened significantly by the identification of CFTR inhibitors by HTS. Building on the observation that $CFTR_{inh}$-172 retarded cyst formation and growth,[122] Yang et al.[123] screened

32 CFTR inhibitors related to $CFTR_{inh}$-172 and GlyH-101 for their ability to block cyst formation and growth. Through these studies, Yang et al.[123] identified tetrazolo-$CFTR_{inh}$-172 and Ph-GlyH-101, two small-molecules that efficaciously inhibit cyst formation and enlargement, without affecting MDCK cell proliferation or inducing apoptosis. When tested using a neonatal kidney organ culture model of cyst formation and growth, both tetrazolo-$CFTR_{inh}$-172 and Ph-GlyH-101 reduced markedly the formation of cysts.[123] Based on these data, Yang et al.[123] investigated the effects of tetrazolo-$CFTR_{inh}$-172 and Ph-GlyH-101 on cyst formation and kidney enlargement *in vivo* using polycystin-1 knockout mice. Of special note, both CFTR inhibitors reduced cyst formation and decreased kidney size in polycystin-1 knockout mice, demonstrating convincingly proof of concept for the use of CFTR inhibitors in ADPKD.[123]

Despite their effectiveness as inhibitors of renal cyst growth,[122,123] thiazolidinones, such as $CFTR_{inh}$-172, have reduced potency in intact cells because their negative charge hinders small-molecule accumulation within the cytoplasm.[124] In search of new CFTR inhibitors with increased potency, Verkman and colleagues screened more than 100,000 small synthetic and natural compounds using their HTS assay. Following electrophysiological evaluation of active compounds, Tradtrantip et al.[124] identified a new class of CFTR inhibitors, pyrimido-pyrrolo-quinoxalinedione (PPQ) compounds. In contrast to other CFTR inhibitors, PPQs are uncharged at physiological pH.[124] The most potent ligand within this chemical class, PPQ-102 inhibited CFTR Cl^- currents by a voltage-independent mechanism that increases channel closed-time without altering current flow through open channels.[124] These data suggest that PPQ-102 is an allosteric inhibitor of CFTR. However, further experiments are required to determine whether its mode of action is similar to $CFTR_{inh}$-172 or genistein.

When tested in polarised epithelia, PPQ-102 inhibited CFTR-mediated transepithelial Cl^- secretion in FRT epithelia expressing recombinant human CFTR with IC_{50} ~90 nM and airway and intestinal epithelia expressing native CFTR with IC_{50} < 1 μM.[124] To begin to explore the therapeutic potential of PPQ-102 in ADPKD, Tradtrantip et al.[124] used their neonatal kidney organ culture model of cyst formation and growth. In this assay, PPQ-102 was more effective than either tetrazolo-$CFTR_{inh}$-172 or Ph-GlyH-101 at preventing cyst formation and growth.[123,124] Because PPQ-102 had poor metabolic stability, Snyder et al.[125] synthesized PPQ and benzopyrimido-pyrrolo-oxazine-dione (BPO) analogues. Like PPQ-102, the most potent small-molecule identified BPO-27 (Fig. 8) was uncharged at physiological pH.[125] However, it exhibited >10-fold metabolic stability than PPQ-102 when tested in hepatic microsomes.[125] Strikingly, BPO-27 inhibited CFTR-mediated transepithelial Cl^- secretion in FRT epithelia expressing recombinant human CFTR with IC_{50} ~8 nM and prevented cyst formation and growth in cultured neonatal kidneys with IC_{50} ~100 nM, a 5-fold improvement over PPQ-102.[124,125] These data argue persuasively that BPO-27 is an excellent lead compound for testing in animal models of ADPKD.

1.5.3 Towards the therapeutic application of CFTR inhibitors

The evaluation of the therapeutic potential of CFTR inhibitors has reached an exciting stage. There is understanding of their mechanism of action, efficacious lead compounds have been tested in animal models and clinical trials of one CFTR inhibitor have been initiated for secretory diarrhoea. But, what of the future? First, it will be important to establish the specificity of lead compounds by testing these small-molecules on a battery of targets, including

ion channels and transporters commonly found in epithelial tissues. Little information is currently available about the specificity of BPO-27,[125] $CFTR_{inh}$-172 appears quite selective for CFTR (Ma et al.[106] but, see Kelly et al.[126]), whereas GlyH-101 is less specific, inhibiting the Ca^{2+}-activated Cl^- channel TMEM16A,[127] SLC26 transporters[128] and various cation channels in cardiac myocytes.[129] Second, it is unknown whether CFTR inhibitors, by themselves, will be an effective therapy for ADPKD. The observation that neither tetrazolo-$CFTR_{inh}$-172 nor Ph-GlyH-101 affected cell proliferation[123] raises the possibility that CFTR inhibitors might need to be given in combination with a small-molecule that inhibits cAMP-stimulated cell proliferation, a key element in the pathogenesis of ADPKD.[40] Third, cyst growth occurs slowly, over decades in ADPKD patients.[37] There is therefore concern that long-term treatment of ADPKD patients with CFTR inhibitors might lead to the development of CF-like symptoms. The toxicity of CFTR inhibitors will therefore need to be evaluated rigorously prior to their use in the clinic. Finally, small-molecule CFTR inhibitors work by inhibiting the activity of individual CFTR Cl^- channels. However, the magnitude of CFTR-mediated Cl^- secretion across the apical membrane of an epithelium is determined both by the number of CFTR channels in the apical membrane and the activity of individual channels (see above, *Section 1.4.4 Towards the therapeutic application of CFTR correctors and potentiators*). Based on the effects of the F508del-CFTR mutation on renal cyst formation and growth *in vivo* and *in vitro*,[130-132] an alternative strategy to retard cyst formation and growth in ADPKD would be to eliminate the apical membrane expression of CFTR. In support of this idea, pioglitazone, a peroxisome proliferator-activated receptor-γ (PPARγ) agonists attenuated sharply CFTR-mediated transepithelial Cl^- secretion by decreasing CFTR mRNA levels *in vitro*[133] and abrogated cyst growth *in vivo*.[134] It will therefore be

interesting to evaluate further the therapeutic potential of pioglitazone and related agents using polycystin-1 knockout mice.

1.6 CONCULSION

The success of ivacaftor in clinical trials demonstrates convincingly that small-molecules that target directly CFTR have significant therapeutic potential. In the case of CF, personalized medicines might be developed to target precisely the genetic defects harboured by individuals afflicted by the disease. Early intervention with these medicines is expected to improve significantly the life expectancy and quality of life of CF patients. In the case of secretory diarrhoea and ADPKD, preclinical studies have demonstrated convincingly the therapeutic promise of CFTR inhibitors. The challenge now is to successfully translate these results into benefits for patients in the clinic. Finally, the success of ivacaftor is a paradigm for the creation of therapeutics targeting the root cause of other genetic diseases.

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FIGURE LEGENDS

Figure 1: Role of CFTR in transepithelial fluid and electrolyte movements (A) CFTR-driven fluid secretion lubricates ducts and tubes. (B) CFTR-mediated Cl^- absorption in sweat duct epithelia. (C) Regulation of ASL by CFTR. See text for further information.

Figure 2: F508del-CFTR disrupts CFTR channel gating Representative recordings of wild-type and F508del-CFTR Cl^- channels in excised inside-out membrane patches from cells expressing recombinant human CFTR. ATP (1 mM) and PKA (75 nM) were continuously present in the intracellular solution, voltage was -50 mV, there was a large Cl^- concentration gradient across the membrane patch ($[Cl^-]_{int}$, 147 mM; $[Cl^-]_{ext}$, 10 mM) and temperature was 37 °C. Dotted lines indicate where channels are closed and downward deflections of the traces correspond to channel openings.

Figure 3: ATP-driven NBD dimerisation opens the CFTR pore The schematic model shows an activated CFTR Cl^- channel. ATP-driven NBD dimerisation opens the CFTR pore. The magnified NBDs show that each ATP-binding site is formed by the Walker A and B motifs (labeled A and B, respectively) of one NBD and the LSGGQ motif of the other NBD. ATP-binding site 2 contains a canonical LSGGQ motif, whereas ATP-binding site 1 contains a non-canonical LSGGQ motif (LSHGH). ATP-binding site 2 also contains a catalytic base (E1371) at the distal end of the Walker B motif, but this residue is absent in site 1 (S573). The location of the CF mutations F508del (surface of NBD1 opposite ICL4), G551D (site 2) and G1349D (site 1) are shown. Abbreviations: MSD, membrane-spanning domain; NBD, nucleotide-binding domain; P, phosphorylation of the RD; P_i, inorganic phosphate; RD, regulatory domain. In and

Out denote the intra- and extracellular sides of the membrane, respectively. See text for further information. Reproduced, with permission, from Chen et al.[135] and Hwang & Sheppard[136].

Figure 4: Chemical structures of clinically-tested CFTR correctors and potentiators Abbreviations: VX-809, 3-{6-{[1-(2,2-difluoro-1,3-benzodioxol-5-yl)cyclopropanecarbonyl]amino}-3-methylpyridin-2-yl}benzoic acid; ivacaftor, N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide.

Figure 5: CFTR potentiators enhance channel gating Representative recordings show the effects of dichlorofluorescein (20 μM), phloxine B (1 μM) and UC_{CF}-853 (10 μM) on the single-channel activity of wild-type CFTR in excised inside-out membrane patches from cells expressing recombinant human CFTR. With the exception that ATP (0.3 mM) was used to support channel activity, experimental conditions were the same as those described in Figure 2. Dotted lines indicate where channels are closed and downward deflections of the traces correspond to channel openings.

Figure 6: Location of some drug-binding sites on CFTR Simplified model of an open CFTR Cl^- channel illustrating the general position of some drug-binding sites. Some small-molecules bind at the NBD dimer interface at a location distinct from the two ATP-binding sites (labeled 1). Other small-molecules dock with a cavity formed by deletion of F508 at the interface of NBD1 and ICL4 (labeled 2). Yet other small-molecules bind at the interface of the NBDs and MSDs involving ICL 1, 2 and 4 (labeled 3). For further information, see text and Figure 3. Modified with permission from Hwang & Sheppard[136] and Sheppard[137].

Figure 7: Blockade of CFTR by open-channel and allosteric inhibitors Representative recordings show the effects of glibenclamide (50 μM) and $CFTR_{inh}$-172 (2 μM) on the single-channel activity of wild-type CFTR in excised inside-out membrane patches from cells expressing recombinant human CFTR. With the exception that ATP (0.3 mM) was used to support channel activity, experimental conditions were the same as those described in Figure 2. Dotted lines indicate where channels are closed and downward deflections of the traces correspond to channel openings.

Figure 8: Chemical structures of some CFTR inhibitors Abbreviations: $CFTR_{inh}$-172, 3-[(3-trifluoromethyl)phenyl]-5-[(4-carboxyphenyl)methylene]-2-thioxo-4-thiazolidinone; GlyH-101, N-(2-naphthalenyl)-[(3,5-dibromo-2,4-dihydroxyphenyl)methylene]glycine hydrazide; iOWH032, 3-(3,5-dibromo-4-hydroxy-phenyl)-N-[(4-phenoxyphenyl)methyl]-1,2,4-oxadiazole-5-carboxamide; BPO-27, 6-(5-bromofuran-2-yl)-7,9-dimethyl-8,10-dioxo-11-phenyl-7,8,9,10-tetrahydro-6*H*-benzo[*b*]pyrimido[4',5':3,4]pyrrolo[1,2-*d*][1,4]oxazine-2-carboxylic acid.

ABSTRACT

The cystic fibrosis transmembrane conductance regulator (CFTR) plays a pivotal role in fluid and electrolyte movements across ducts and tubes lined by epithelia. Loss of CFTR function causes the common life-limiting genetic disease cystic fibrosis (CF) and a spectrum of disorders termed CFTR-related diseases, while unphysiological CFTR activity characterises secretory diarrhoea and autosomal dominant polycystic kidney disease (ADPKD). The prevalence of these disorders argues persuasively that small-molecule CFTR modulators have significant therapeutic potential. Here, we discuss how knowledge and understanding of the CFTR Cl^- channel, its physiological role and malfunction in disease led to the development of the CFTR potentiator ivacaftor, the first small-molecule targeting CFTR approved as a treatment for CF. We consider the prospects for developing other therapeutics targeting directly CFTR including CFTR correctors to rescue the apical membrane expression of CF mutants, CFTR corrector-potentiators, dual-acting small-molecules to correct the processing and gating defects of F508del-CFTR, the commonest CF mutant and CFTR inhibitors to prevent fluid and electrolyte loss in secretory diarrhoea and cyst swelling in ADPKD. The success of ivacaftor provides impetus to other CFTR drug development programmes and a paradigm for the creation of therapeutics targeting the root cause of other genetic disorders.

Keywords: ATP-binding cassette transporter / epithelial ion transport / cystic fibrosis / CFTR / chloride ion channel / channel gating / F508del / CFTR corrector / CFTR potentiator / CFTR inhibitor

Figure 1

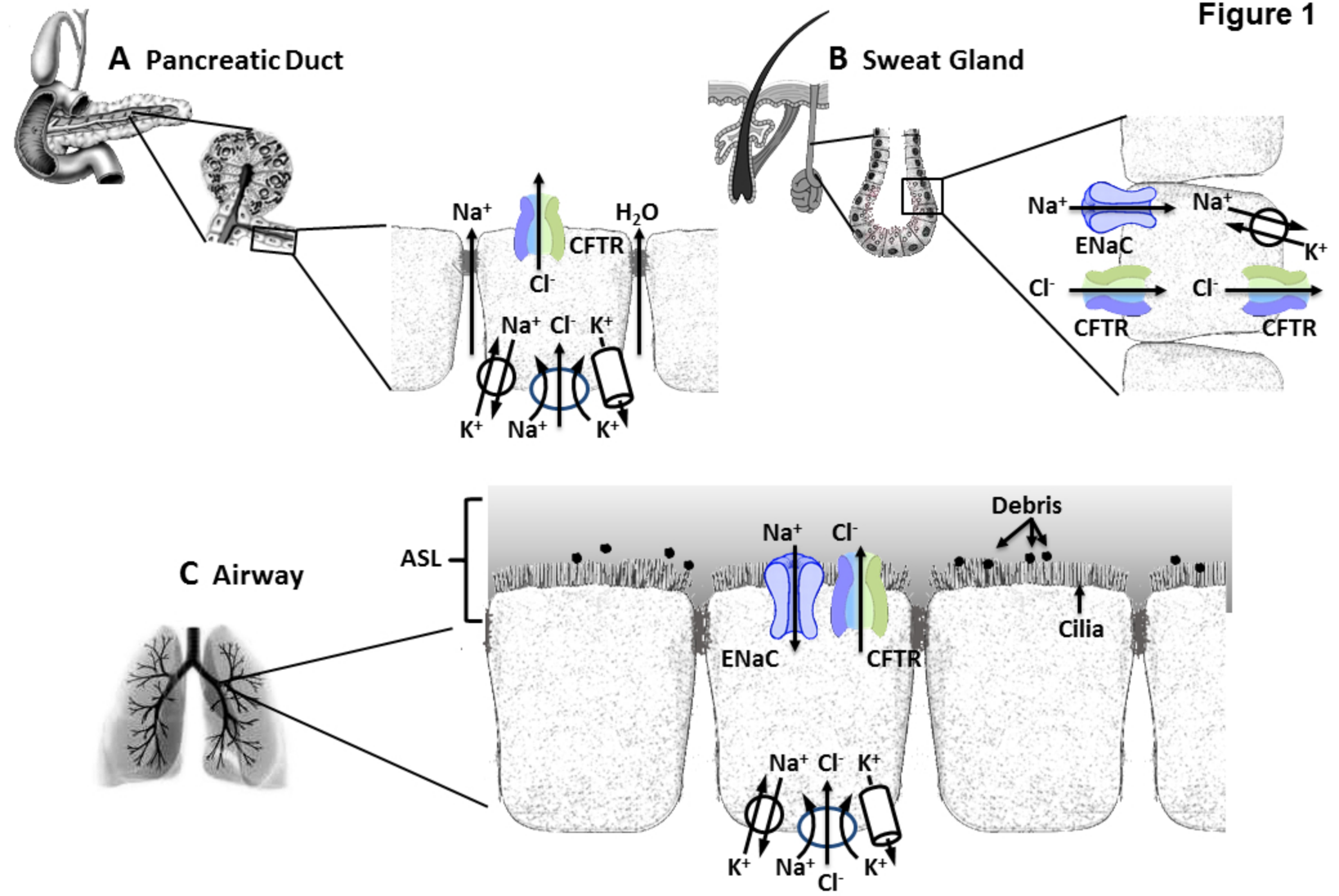

A Pancreatic Duct
Na+
H2O
CFTR
Cl-
Na+ Cl- K+
K+ Na+ K+
B Sweat Gland
Na+
ENaC
Na+
K+
Cl-
CFTR
Cl-
CFTR
C Airway
ASL
Debris
Na+
Cl-
ENaC
CFTR
Cilia
Na+ Cl- K+
K+ Na+ Cl- K+

Figure 2

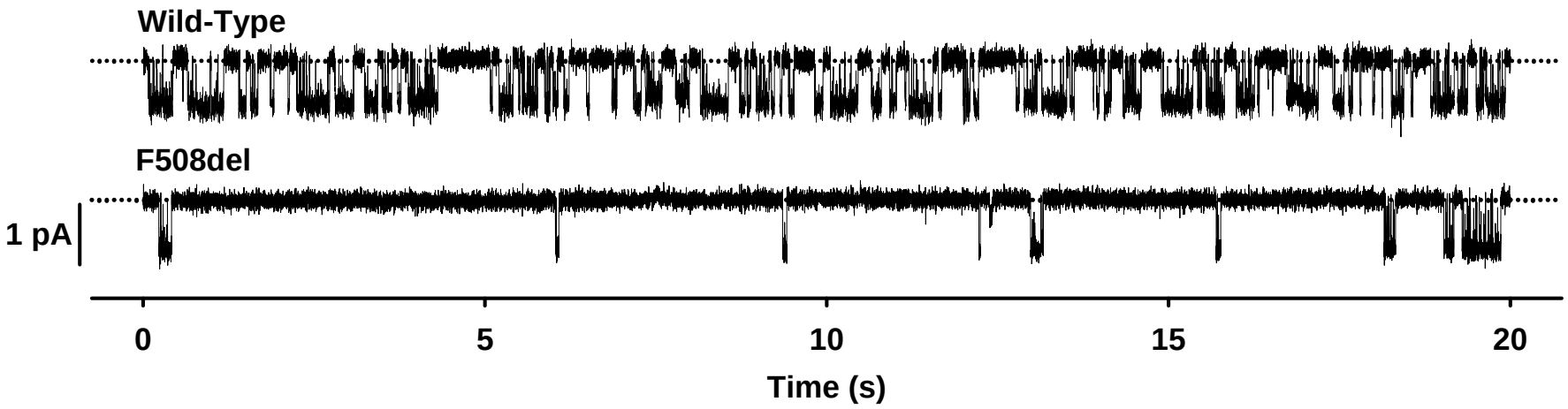


Figure 3

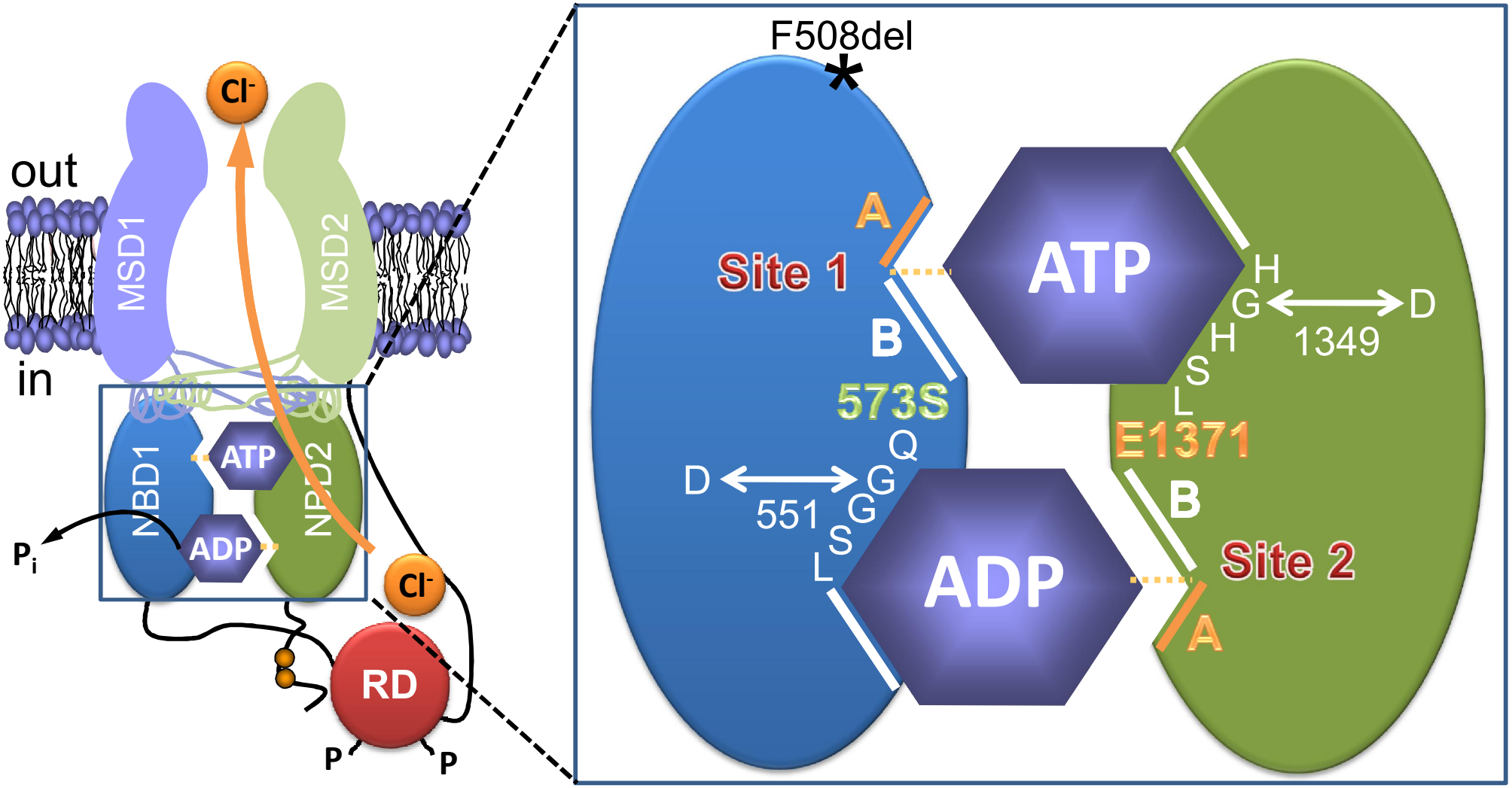
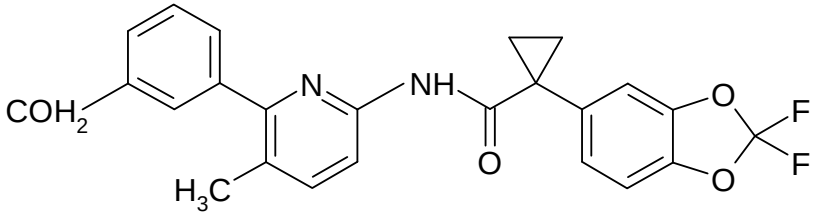
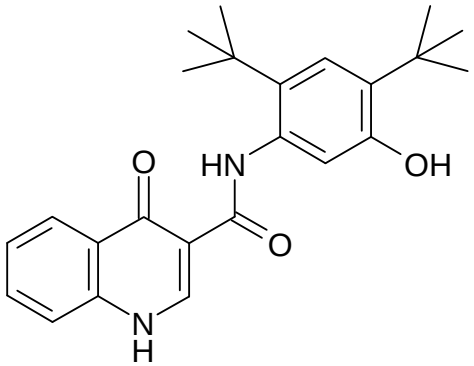

out
in
MSD1
MSD2
NBD1
NBD2
ATP
ADP
Cl-
Cl-
Pi
RD
P
P
F508del
*
Site 1
A
B
573S
Q
G
G
S
L
D
551
ATP
ADP
H
G
D
1349
H
S
L
E1371
B
Site 2
A

Figure 4



VX-809



VX-770

Figure 5

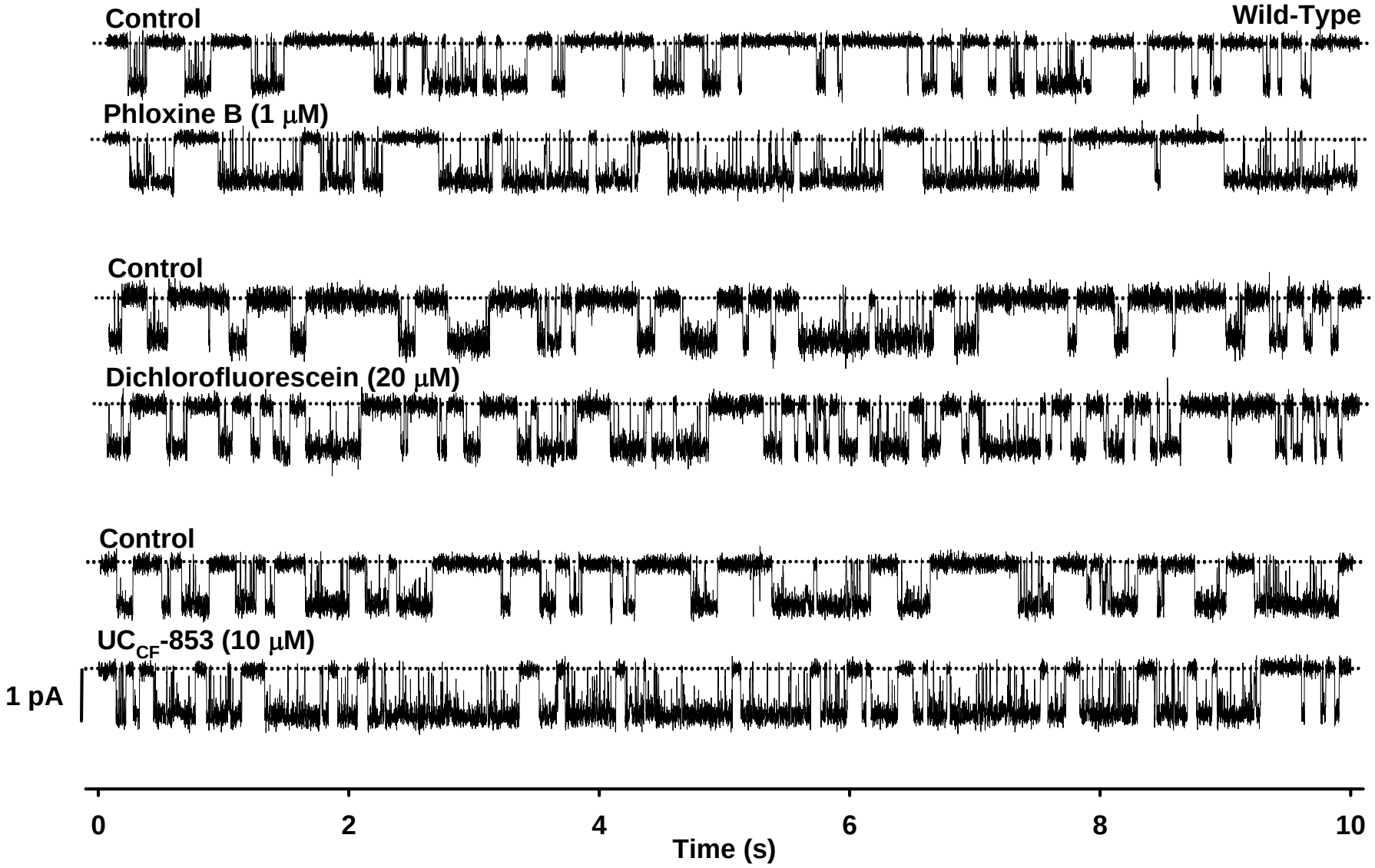

Control
Wild-Type
Phloxine B (1 μM)
Control
Dichlorofluorescein (20 μM)
Control
UCCF-853 (10 μM)
1 pA
0
2
4
6
8
10
Time (s)

Figure 6

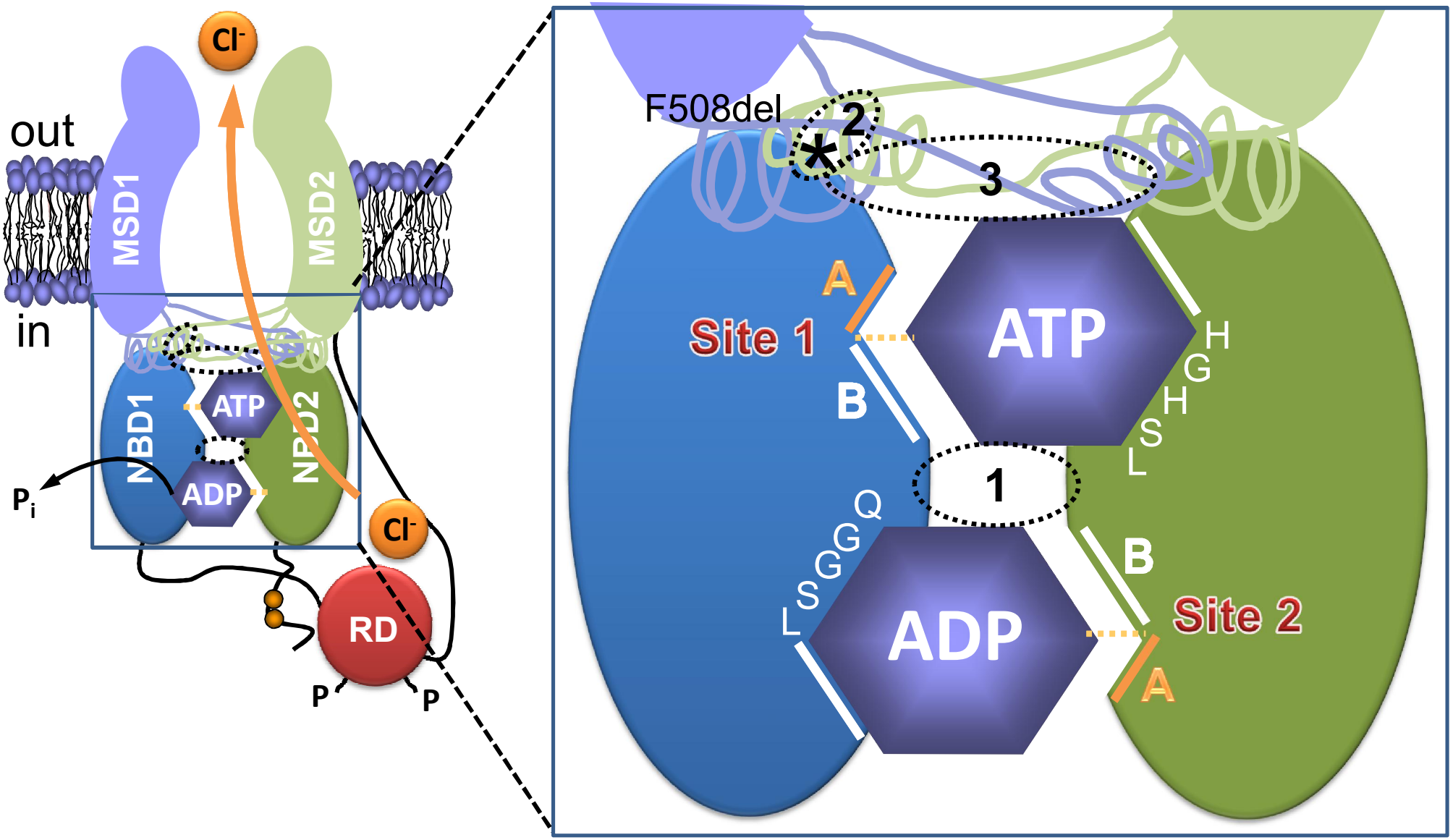

out
in
MSD1
MSD2
NBD1
NBD2
ATP
ADP
Pi
Cl-
Cl-
RD
P
P
F508del
2
3
1
A
B
Site 1
ATP
H
G
H
S
L
Q
G
G
S
L
ADP
B
Site 2
A

Figure 7

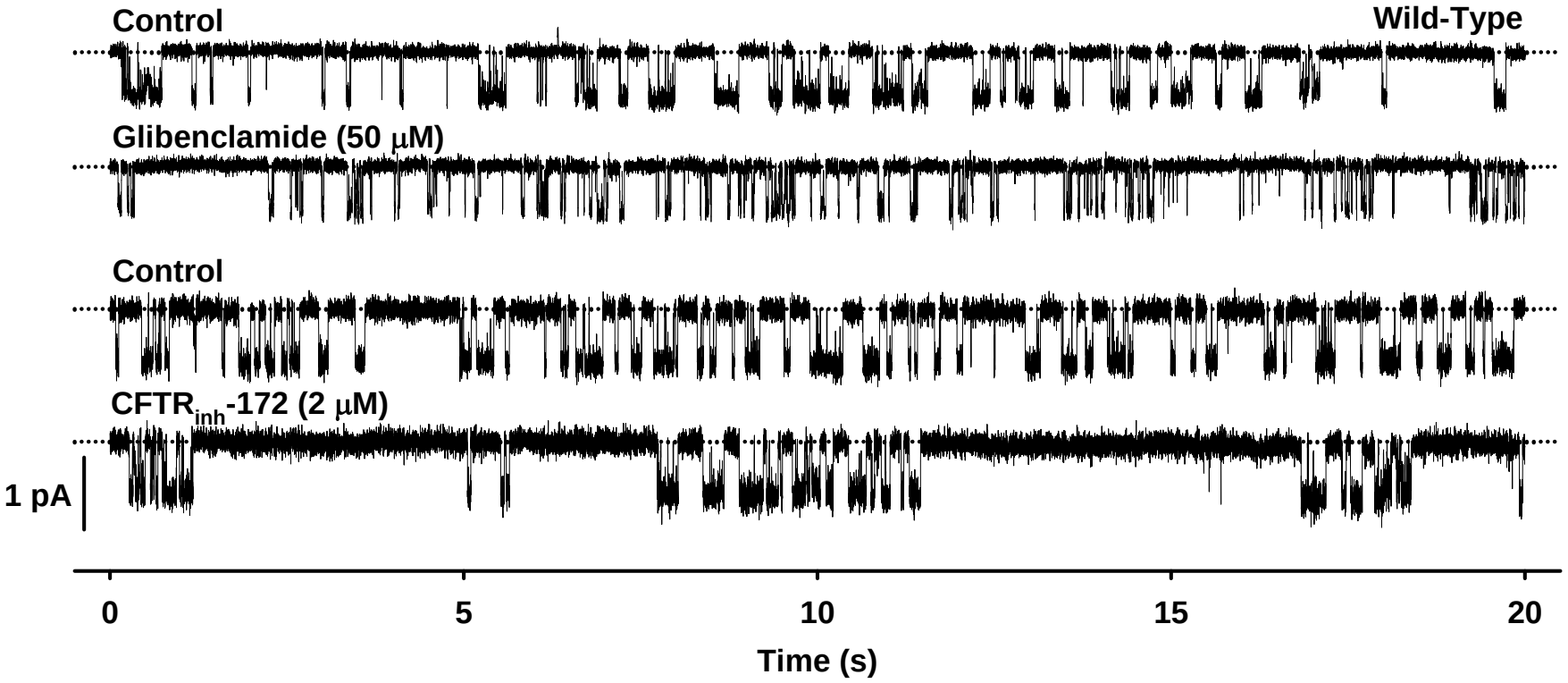
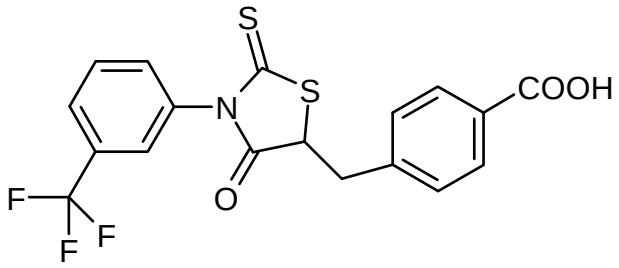
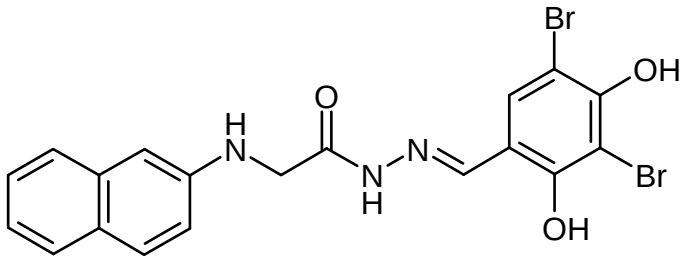

Control
Wild-Type
Glibenclamide (50 μM)
Control
CFTRinh-172 (2 μM)
1 pA
0
5
10
15
20
Time (s)

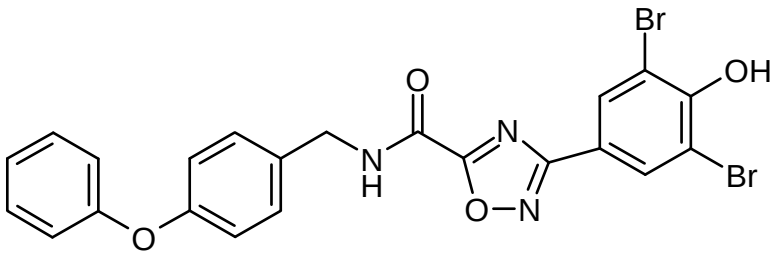
Figure 8



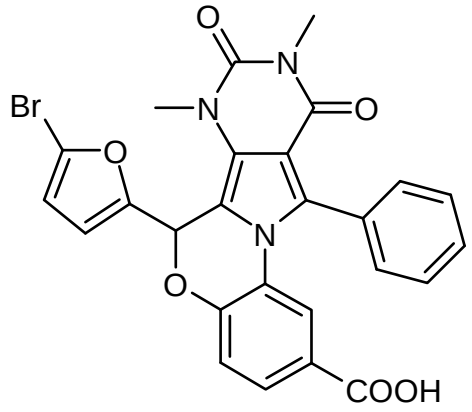
$CFTR_{inh}$-172



GlyH-101



iOWH032



BPO-27